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High *Triatoma brasiliensis* Densities and *Trypanosoma cruzi* Prevalence in Domestic and Peridomestic Habitats in the State of Rio Grande do Norte, Brazil: The Source for Chagas Disease Outbreaks?

Mauricio Lilioso,[1*] Elaine Folly-Ramos,[1] Fabiana Lopes Rocha,[1] Jorge Rabinovich,[2] Claire Capdevielle-Dulac,[3] Myriam Harry,[3] Paula L. Marcet,[4] Jane Costa,[5†] and Carlos Eduardo Almeida[1,6*†]

[1]*Programa de Pós-Graduação em Ecologia e Monitoramento Ambiental (PPGEMA), Universidade Federal da Paraíba (UFPB), Joao Pessoa, Brazil;* [2]*Centro de Estudios Parasitológicos y de Vectores (CEPAVE), Universidad Nacional de La Plata, La Plata, Argentina;* [3]*UMR Evolution, Génomes, Comportement, Ecologie, CNRS-IRD-Univ. Paris-Sud, Université Paris Saclay, Paris, France;* [4]*Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia;* [5]*Laboratorio de Biodiversidade Entomologica, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil;* [6]*Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil*

Abstract. A total of 2,431 *Triatoma brasiliensis* were collected from 39 populations of Paraíba (PB) and Rio Grande do Norte (RN) states, Brazil. In PB, *Trypanosoma cruzi* infection was not detected in either peridomestic or domestic vector populations. In contrast, in RN, *T. brasiliensis* was detected with high parasite prevalence in these ecotopes (30.7–40.0%). Moreover, peridomicile insect population densities were more than double the average densities of all other settings evaluated (19.17 versus < 8.94 triatomine/man-hour). Genotyped parasites evidenced a mix of *T. cruzi* lineages circulating in both peridomestic and sylvatic populations. Although vector control efforts have dramatically decreased Chagas disease transmission to humans, recent outbreaks have been detected in four municipalities of RN state. Our results clearly evidence a worrisome proximity between infected vectors and humans in RN. Indeed, finding of infected *T. brasiliensis* inside homes is routinely recorded by local vector control surveillance staff around the outbreak area, challenging the current and conventional view that vector transmissions are controlled in northeastern Brazil. This scenario calls for strengthening vector control surveillance and interventions to prevent further Chagas transmission, especially in RN State.

INTRODUCTION

Before the implementation of massive vector control actions under the Southern Cone initiative, *Triatoma infestans* had been considered the major Chagas disease vector across most Brazilian regions. However, this species had never been the main vector in endemic states of northeastern Brazil, such as Paraíba (PB), Ceará, and Rio Grande do Norte (RN).[1,2] The largest serological survey ever conducted for endemic chagasic infections in rural human populations took place between 1975 and 1980,[3] when *T. infestans* was widespread in Brazil, and placed PB state in the eighth (3.4%) and RN in the 15th (1.8%) positions in a nationwide prevalence assessment. In RN, hyperendemic transmission of Chagas disease was reported in 1957 and 1962,[4] with estimations of 12.2% human infections in areas where 65.1% of captured insects were *Triatoma brasiliensis* Neiva, 1911. Moreover, domiciliary populations of *T. brasiliensis* from RN were shown to exhibit the highest natural *Trypanosoma cruzi* infection prevalence (4.5%) in the Brazilian northeast.[5] Several studies also showed that *T. brasiliensis* invades and colonizes domiciles, usually around 6 months after insecticide residual spraying for vector control.[6–9]

In RN state, sylvatic *T. brasiliensis* populations have been found with high *T. cruzi* prevalence (51–72%), and active gene flow with domestic populations was demonstrated.[10] In view of this scenario, we assessed *T. cruzi* infection rates in domestic, peridomestic, and sylvatic *T. brasiliensis* populations in RN and also in PB state, where gene flow between sylvatic and domestic populations has also been reported.[11] Additionally, *T. cruzi* from two ecotypic populations were genotyped to determine the lineages circulating in the area.

*Address correspondence to Carlos Eduardo Almeida, Universidade Estadual de Campinas, Unicamp, Cidade Universitária Zeferino Vaz - Barão Geraldo, Campinas - SP, Brazil, 13083-970, E-mail: almeida_ce@hotmail.com or Mauricio Lilioso, Programa de Pós-Graduação em Ecologia e Monitoramento Ambiental (PPGEMA), Centro de Ciências Aplicadas e Educação, Universidade Federal da Paraíba (UFPB), Rua da Mangueira s/n. Centro, Rio Tinto, Paraíba 58297-000, Brazil, E-mail: or mauriciolilioso@hotmail.com.
†Co-last authors.

STUDY AREA

The study was conducted in the semiarid region of the states of PB and RN, within the geographic distribution of *T. brasiliensis*.[12] We divided the sampling area into four geographic districts: Cajazeiras (CZ) and Patos (PA) in PB state and Currais Novos (CN) and Caicó (CC) from RN state (Figure 1).

EXPERIMENTAL DESIGN

We conducted an entomological evaluation in domiciliary units (DUs, which includes the domicile and/or the peridomicile ecotopes). The sample size comprised 7–8 DUs per district. Parasite molecular characterization was conducted in the municipalities where *T. cruzi* was detected via microscopy in triatomine feces (see below) in populations from both DU and sylvatic environments within 1 km from each other.

INSECTS

Triatomine collections were conducted from September 2014 to March 2015. Insects were sorted according to the ecotope of capture: domiciliary, peridomiciliary, and sylvatic.[13] The sylvatic environments were rocky outcrops, considered the primary sylvatic ecotope of *T. brasiliensis*.[14]

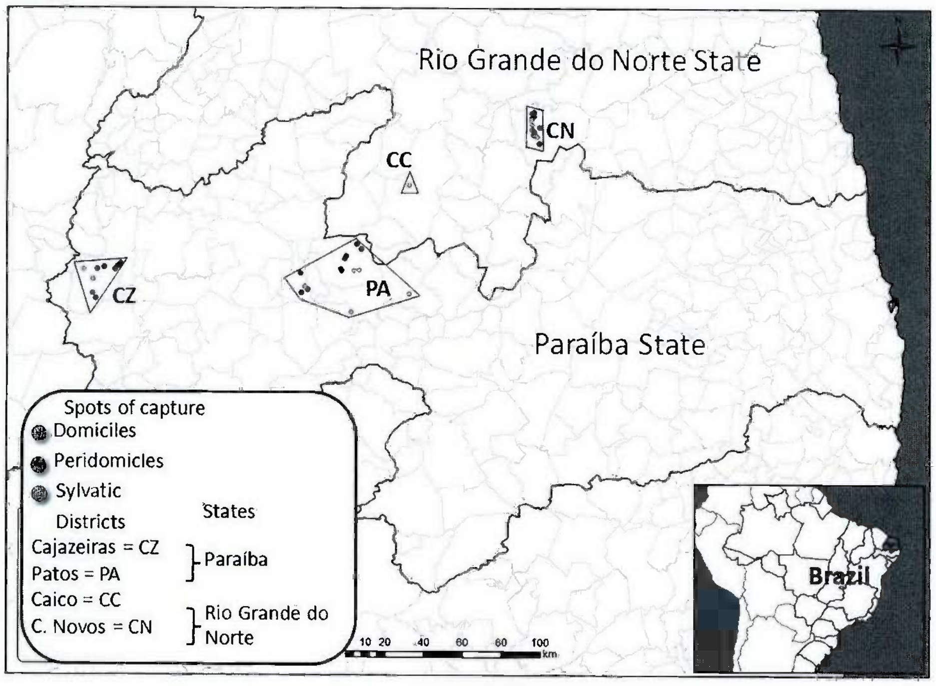

Figure 1. Spots of capture of *Triatoma brasiliensis* and their respective *Trypanosoma cruzi* prevalence per study site in the states of Paraíba and Rio Grande do Norte, Brazil. Districts: CZ = Cajazeiras, PA = Patos, CC = Caicó, and CN = Currais novos. This figure appears in color at www.ajtmh.org.

Domiciliary ecotopes were the indoor space of houses, where bug searches were carried out with the aid of local vector control surveillance staff, looking under stoves, beds, between spaces of the roof, behind wall-placed pictures, and among stored belongings (food, clothes, home materials). In peridomiciles, most triatomines were collected in storerooms, henhouses, corrals, pigsties, and piles of tiles, bricks, or stones, among others. All insects were captured with tweezers without irritant product to dislodge insects. In the DUs, we used the method of capture by exhaustion: collectors remained in the site until no more bugs were visible. The final time spent in each DU was recorded (Supplemental File 1). In the sylvatic environments, a period of 4 hours was established to search and capture. Triatomine density was estimated as the number of captured insects per man per hour.[13] Taxonomic morphological identification was carried out according to the newest taxonomic key.[15]

DETECTION OF *T. CRUZI* INFECTION

Parasite prevalence in *T. brasiliensis* was evaluated as follows: one fecal drop from each bug was obtained by abdominal compression, then diluted in saline solution (approximately 50 μL), and examined by microscopy at 220–400× by highly trained personnel.

PARASITE MOLECULAR CHARACTERIZATION

DNA extraction from the digestive tract of individual insects was carried out with the DNeasy Tissue Kit (Qiagen, Hilden, Germany). The nontranscribed intergenic region of *T. cruzi* miniexon gene was amplified in a multiplex reaction using three primers (TCI/TCII/TC) that amplify the two main *T. cruzi* subtypes.[16] TCI is specific to the DTU TcI generating a fragment of 350 base pairs (bp), and TCII amplifies DTUs TcII, TcV, and TcVI, with a polymerase chain reaction (PCR) product of 300 bp. DNA from *Rhodnius prolixus* experimentally infected with TcVI (CL Brener strain) was used as a positive control for the 300-bp product, and water as the negative. PCR amplification was carried out as previously described,[16] and amplifications were visualized in 1% agarose gel stained with ethidium bromide. Parasites herein genotyped were called TCI and TCII*.

A total of 2,431 *T. brasiliensis* were collected, comprising 39 populations, according to the ecotope and capture site: 11 in CZ (five domiciliary, four peridomiciliary, and two sylvatic), 12 in PA (two domiciliary, five peridomiciliary, and five sylvatic), 13 in CN (one domiciliary, eight peridomiciliary, and four sylvatic), and three in CC (all sylvatic) districts (Table 1). Nymphs were present in almost all collection sites, which was a robust indication of colonization. Supplemental File 1 shows details about each population, including population location, developmental stage, and *T. cruzi* infection results. A total of 792 *T. brasiliensis* were analyzed for *T. cruzi* infection. In PB state, only the sylvatic populations had *T. cruzi*-positive bugs, with an overall prevalence of 6.3% (4/63) in the CZ district and 1.6% (2/129) in PA. None of the peridomestic or domestic insect populations in CZ (N = 143) and PA (N = 100) were infected. The situation was different in RN state. In the CN district, 30.7% (54/176) and 40.0% (2/5) insects of the peridomestic and domestic ecotopes were infected with *T. cruzi*. Remarkably, one peridomestic population, named CN76P, exhibited 86.7% (26/30) infection rate (Supplemental File 1). A similar profile of *T. cruzi* prevalence was observed for the sylvatic environment, with 33.8% (25/74) infected insects. In CC, *T. cruzi* prevalence

Table 1

Triatoma brasiliensis survey in the states of Paraíba and Rio Grande do Norte, Brazil

	Districts	Ecotopes	Pops	M	F	N5	N4	N3	N2	N1	T(r)	Inf. %	An.	Inf. N
Paraíba State	CZ	Domestic	5	7	12	19	6	7	5	1	57 (2.25)	0.0%	57	0
		Peridomestic	4	9	23	34	15	3	1	1	86 (3.97)	0.0%	86	0
		Sylvatic	2	47	30	13	15	6	2	0	113 (4.71)	6.3%	63	4
	PA	Domestic	2	0	2	3	2	3	8	5	23 (2.39)	0.0%	23	0
		Peridomestic	5	24	29	65	46	24	8	2	198 (6.19)	0.0%	77	0
		Sylvatic	5	69	47	41	21	16	3	0	197 (3.28)	1.6%	129	2
Rio Grande do Norte State	CN	Domestic	1	0	1	3	6	3	0	0	13 (3.25)	40.0%	5	2
		Peridomestic	8	92	48	313	380	299	63	12	1,207 (19.17)	30.7%	176	54
		Sylvatic	4	75	24	89	94	29	7	4	322 (8.94)	33.8%	74	25
	CC	Sylvatic	3	91	28	58	29	9	0	0	215 (5.97)	72.5%	102	74
		General Total	39	414	244	638	614	399	97	25	2,431 (7.50)	20.3%	792	161

CC = Caicó; CN = Currais Novos; CZ = Cajazerias; PA = Patos. The survey includes the ecotopes, number of populations sampled (Pops), evolutionary stages (M = males, F = females, N = stages), total of bugs collected (T = total number of captured bugs and r = rate of bugs captured per man per hour), prevalence of *Trypanosoma cruzi* natural infection (Inf. %), total of analyzed bugs (An.), and total of infected bugs (Inf. N) per each geographic district sampled. Fieldworks were conducted from September (2014) to March (2015). Details about each population are in the Supplemental File 1.

was 72.2% (74/102) for the sylvatic insect population, confirming previous findings of high natural *T. cruzi* prevalence in the area.[10] Higher triatomine density was found in the peridomestic populations from CN, resulting in 19.7 bugs captured/man-hour, whereas all other ecotypic populations had capture rates below half of it (< 8.9 bugs captured/man-hour, Table 1).

Molecular typing for the miniexon gene of *T. cruzi* was carried out for only one population pair in CN, where parasites were detected in both, sylvatic and DUs located near each other (575 m apart). The sylvatic population CN83S exhibited 50% of *T. cruzi*-infected insects (10/20), nine of which were TCII* and one that had mixed infection (TCI/TCII*). In the peridomiciliary population CN69P, 25% (5/20) of the insects were infected: one harbored TCI, two TCII*, and two exhibited a mix of TCI/TCII*. These results also confirm that the parasites identified via microscopy were in fact *T. cruzi*. The cooccurrence of TCI and TCII* in the same ecotope, the prevalence of TCII*, as well as mixed infections within bugs, are similar to those observed in CC,[10] situated 68.5 km away from CN. Because TCII* can be three different DTUs (TcII, TcV, and TcVI), to characterize the gene flow among ecotopes and or hosts, detailed parasitic genotyping, including complete DTU characterization and individual strain typing should be considered in future studies.

The combination of high *T. cruzi* prevalence and high *T. brasiliensis* densities in the peridomestic environment in CN municipality from RN is a real threat, which together with the presence of infected bugs inside houses increases our concern. Moreover, *T. cruzi* prevalence was higher in the sylvatic environment in all municipalities of RN, which may be related with wild reservoirs, as has been shown in by Almeida and others[10] that insects infected by *T. cruzi*, had also fed on native rodents of the Caviidae family, as the rocky cavy (*Kerodon rupestris*) and with Spix's yellow-toothed cavy (*Galea spixii*) in RN.

In 2015, outbreaks of Chagas disease transmission were officially recorded in four municipalities of RN with 14 confirmed human cases and some more still awaiting laboratory and clinical confirmation.[17] Herein, we present evidence of a clear proximity between *T. cruzi*-infected vectors and humans in the same state where the outbreaks have been recorded, and a report alerting for the hazard was sent to those involved in vector control. Although it is not known if human cases were caused by vector or oral transmissions, vector involvement is crucial for keeping the parasitic cycle. Finding infected *T. brasiliensis* inside homes in CN municipality (RN), is indeed, routinely recorded by local vector control-surveillance staff (J. T. Santos, personal communication; Supplemental File 2), challenging the current and conventional view that vector transmissions are controlled in northeastern Brazil. Further studies with higher resolution molecular markers (e.g., microsatellites) could individualize *T. cruzi* individual strains and help determine whether the strains involved in the outbreak observed in RN are related to those circulating among *T. brasiliensis* populations in the area.

The levels of house infestation, the presence of sylvatic population in proximity of the houses, as well as the high prevalence of *T. cruzi* within *T. brasiliensis* in the region, call to further recognize the epidemiological importance of this vector and to implement aggressive vector control strategies targeting this species. Additionally, we recommend a multisource study, combining eco-epidemiological[18,19] and geospatial[20] analyses to better understand the factors related to the risk presented by *T. brasiliensis* to transmit *T. cruzi* to humans.

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Authors' addresses: Mauricio Lilioso, Elaine Folly-Ramos, and Fabiana Lopes Rocha, Programa de Pós-Graduação em Ecologia e Monitoramento Ambiental (PPGEMA), Centro de Ciências Aplicadas e Educação, Universidade Federal da Paraíba (UFPB), Joao Pessoa, Brazil, E-mails: mauriciolilioso@hotmail.com, elafolly@yahoo.com.br, and lopesrocha.fabiana@gmail.com. Jorge Rabinovich, Entomologya, Centro de Estudios Parasitologicos y de Vectores, Universidad Nacional de La Plata, La Plata, Argentina, E-mail: jorge.rabinovich@gmail.com. Claire Capdevielle-Dulac and Myriam Harry, Evolution, Génomes, Comportement, Ecologie, Centre National de la Recherche Scientifique, Paris, France, E-mails: claire.capdevielle-dulac@egce.cnrs-gif.f and myriam.harry@u-psud.fr. Paula L. Marcet, Entomology, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, E-mail: pvm3@cdc.gov. Jane Costa, Laboratorio de Biodiversidade Entomologica, Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro, Brazil, E-mail: jcosta@ioc.fiocruz.br. Carlos Eduardo Almeida, Universidade Estadual de Campinas, Unicamp, Cidade Universitária Zeferino Vaz - Barão Geraldo, Campinas - SP, Brazil, 13083-970, E-mail: almeida_ce@hotmail.com.

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